DATA EVALUATION RECORD

(S)-Methoprene – [Isopropyl (2E,4E,7S)-methoxy-3,7,11-trimethyl-2,4-dodecadienoate] (RF-2016 MT)

STUDY TYPE: Premise Treatments (OPPTS 810.3500)

MRID 46163001

Study Title: Residual effectiveness of RF2016 and RF433A against mosquitos

Prepared for
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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

DATA EVALUATION RECORD

EPA Secondary Reviewers:	Angela L. Gonzales and Russell S. Jones
STUDY TYPE:	Premise Treatments (OPPTS 810.3500)
MRID NO:	46163001
DP BARCODE NO:	303194
DECISION NO:	341588
SUBMISSION NO:	
TEST MATERIAL:	(S)-Methoprene – [Isopropyl (2E,4E,7S)-methoxy-3,7,11-trimethyl-2,4-dodecadienoate] (8.62%)
PROJECT NO:	
SPONSOR:	Wellmark International
TESTING FACILITY:	Florida A&M University
TITLE OF REPORT:	Residual effectiveness of RF2016 and RF433A against mosquitos
AUTHORS:	Tom Floore
STUDY COMPLETED:	12/10/03
GOOD LABORATORY PRACTICE:	Not GLP Compliant

SUMMARY: An efficacy study for the registration of the end-use product (EP), RF2016 MT (EPA Registration No. 2724-UOO) was submitted in MRID 46163001. The study was performed in order to establish the effectiveness of this (S)-Methoprene product against *Culex quinquefasciatus* larvae when used in screen enclosed simulated catch basin freshwater plots. RF433A, another (S)-Methoprene formulation, and Valent VectoLex® WSP (*Bacillus sphaericus*), a biological larvicide water soluble pouch were also evaluated with RF2016 MT in this study. This report will primarily focus on the efficacy results for the product in review for registration. Two untreated plots were used as controls and two plots were treated with RF2016 MT. The briquets were placed one per plot and approximately 800 laboratory-reared larvae were added to each plot ten times during the 118-day study. Percent emergence inhibition (%EI) was determined and evaluated at ten time points during the study. At post-treatment day 14, the day of the second evaluation of %EI, the plots were all drained and then reflooded four days later and the evaluations continued until day 118. Bioassay observations and statistics were performed in order to ascertain differences in efficacy between the control groups and the RF2016 MT groups. It is reported in the study that one or both of the plots treated with the product resulted in greater than 95% emergence inhibition throughout the entire experiment. However, there are significant deficiencies throughout the study and many aspects are unclear and need to be clarified and explained.

CLASSIFICATION: SUPPLEMENTAL, upgradable to acceptable pending sufficient revisions, submission of additional information, and clarification of deficiencies listed below.

Test Material: RF2016 MT containing 8.62% (S)-Methoprene – [Isopropyl (2E,4E,7S)-methoxy-3,7,11-trimethyl-2,4-dodecadienoate]

I. MATERIALS AND METHODS

Test Substance: RF2016 MT (EPA Reg. No. 2724-UOO) containing 8.62% (S)-Methoprene – [Isopropyl (2E,4E,7S)-methoxy-3,7,11-trimethyl-2,4-dodecadienoate] (EPA Reg. No. 2724-442, Chemical No. 105402), Lot No. 302121202-A.

Test System: The laboratory used to test the efficacy of RF2016 MT was the same laboratory that has been used (as reported in the study) for many years to test the product performance of the registrant's other registered (S)-Methoprene products. Two untreated plots were used as controls and two plots were used for testing the product. Each plot consisted of an uncovered concrete tank filled with well water, which were all enclosed within a screen. No dirt or vegetation was planted in the plots. A photograph was provided of a plot area. Water temperature during the study ranged from 89.5° to 53.2°F, pH ranged from 7.2 to 10.7, and monthly rainfall ranged from 2.5 to 8.87 inches without flooding any of the plots. The briquets were applied one per plot. At day 14, post-treatment, the plots were drained and allowed to dry for four days at which time they were reflooded and more larvae were reintroduced. There were eight evaluation periods after this event. The study was concluded on day 118.

Methods: During the study, approximately 800 laboratory-reared 2nd and 3rd instar susceptible mosquito larvae were added to each plot ten times. During each evaluation period, about 100 pupae were obtained from each plot using a standard dipper and held in about 30 ml of aerated well water in Styrofoam cups. The cups were then placed in an enclosed porch to allow for the completion of development or death in order to determine %EI.

%EI was calculated by the following formula:

$$\%EI = 100 - [(CS-DA)/(CS+PE+DP^*)] \times 100$$

where "CS" is the number of cast pupae skins, "DP" the number of dead pupae, "PE" the number of partially emerged adults, and "DA" the number of dead adults. The data were then subjected to statistical analysis.

*In the study, DF is listed in the equation instead of DP. The reviewer has assumed that this is a typographical error, and that DF should be DP.

III. RESULTS AND CONCLUSIONS

The results of the study of the %EI of mosquitos from the use of RF2016MT are summarized in Table 1 below:

Table 1.

Plot	Day (post-treatment)	DP	PE	DA	CS	%EI
Control	7	30	6	1	220	14.39
RF2016 MT (a)	7	127	0	0	0	100.00
RF2016 MT (b)	7	121	0	0	0	100.00
Control	14	24	4	1	173	14.49
RF2016 MT (a)	14	177	7	2	8	96.88
RF2016 MT (b)	14	119	1	0	0	100.00

Plot	Day (post-treatment)	DP	PE	DA	CS	%EI
Control	34	11	0	0	191	6.19
RF2016 MT (a)	34	129	1	0	0	100.00
RF2016 MT (b)	34	122	1	0	0	100.00
Control	43	6	1	2	236	3.67
RF2016 MT (a)	43	125	0	0	0	100.00
RF2016 MT (b)	43	100	8	7	2	100.00
Control	50	9	0	0	230	3.76
RF2016 MT (a)	50	125	0	0	0	100.00
RF2016 MT (b)	50	86	7	10	21	90.35
Control	64	20	4	1	213	10.47
RF2016 MT (a)	64	113	7	0	3	97.56
RF2016 MT (b)	64	109	10	1	5	96.77
Control	78	2	3	0	255	1.91
RF2016 MT (a)	78	96	23	6	7	99.21
RF2016 MT (b)	78	47	18	12	45	70.00
Control	92	2	2	0	265	1.57
RF2016 MT (a)	92	108	0	0	0	100.00
RF2016 MT (b)	92	46	3	17	68	56.41
Control	105	2	0	0	265	0.75
RF2016 MT (a)	105	119	1	3	9	95.35
RF2016 MT (b)	105	25	1	2	28	51.85
Control	118	6	1	2	251	3.50
RF2016 MT (a)	118	112	8	3	1	100.00
RF2016 MT (b)	118	117	0	0	0	100.00

It was suggested in the study that the differences in %EI between the two RF2016 MT plots could be attributed to algae blooms in the plots that could have prevented the dispersion of the methoprene throughout the water or bound up the chemical. It was reported that there were algal blooms present in all of the study plots at one time or another, but that the algae did not appear to inhibit larval growth. A reduction in the bloom was noted toward the end of the study period, and it is suggested that this might explain why there was increased control at the end of the study. It was also stated in the report that the observation of differences between plots have occurred for several years and that each plot tends to have its own micro habitat with differences in vegetation density and micro fauna and flora. Since there was no addition of vegetation to the water in this study, there were primarily differences in micro fauna and flora. There was an algal mass that

formed in the RF2016 MT (b) plot. As reported in the study, in the RF2016 MT (a) plot, the product never exhibited less than 90% control. The methoprene briquet in the RF2016 MT (b) plot exhibited less than 90% control in three of the ten evaluation periods.

IV. REVIEWER CONCLUSIONS

This study (MRID 46163001) is classified as supplemental for the following reasons:

1. The registrant must define the endpoint selection, specifically a more precise definition and explanation of emergence and the percent emergence inhibition (%EI), and not solely in the form of an equation. It is unclear what the registrant considers to be "emergence inhibition". For example, on day 43, both plots were reported to have 100% EI, but in one of the plots, dead pupae (DP), partially emerged adults (PE), dead adults (DA) and cast pupae skins (CA) were observed and counted while in the other plot there were none observed. The reviewer would assume that if DP, PE, DA, and CA were counted in a plot, then the EI would not be 100%.

2. In the study, the plots are drained on day 14 and refilled four days later. It is unclear why this was done and no explanation was provided in the study. The registrant is required to clarify why this action was performed, as it does not support label claims.

3. The pH recorded in the study ranged from 7.2 to 10.7. No explanation was given as to why to pH rose to 10.7, an abnormally high pH for the conditions described in the experiment. Additionally, an explanation must be provided by the registrant regarding whether this high pH could or effect the results of the study.

4. In the study, the product was tested in a water surface area measuring approximately 8 square feet with a water depth of 6 inches in each plot, but the label language claims that one briquet can treat up to 100 square feet of water surface area. No data were provided that suggest that RF2016 MT is efficacious in a water surface area of 100 square feet. The label also claims that one briquet can treat up to 1,500 gallons of water, but no data were provided to justify this statement either. Data will be required in order to support these label statements.

5. It is reported in the study that testing was conducted in the uncovered tanks that were within an outdoor screened enclosure. The reviewer assumes that all of the uncovered tanks were within the same outdoor screened enclosure, which introduces the issue of cross-contamination between tanks. There is no information provided in the study discussing whether any cross-contamination occurred. Additional information on this matter is required.

6. The equation used in the study (page 7) to determine %EI was:

$$\%EI = 100 - [(CS-DA)/(CS+PE+DF)] \times 100$$

It was assumed by the reviewer that "DF" is a typographical error and that "DF" should be replaced by "DP". The registrant needs to confirm that this is an accurate assumption.

7. The issue of the algae effect on the effectiveness of RF2016 must be resolved. It states on page 7 of the report, "The algae bloom seem not to be a factor in with the RF2016 formulations" and on page 8 of the report it states, "The algae bloom occurred in all the study plots at one time

or the other but did not appear to inhibit larvae growth. One or a combination of these occurrences might have interfered with the effectiveness of a formulation in a plot during an assessment period." These two statements are contradictory. The registrant needs to provide clarification and information regarding the effect of algae, which as this study proves, is generally present in standing water, on the efficacy of the product for which registration has been requested.

8. No dates or exact time periods were given in the study as to when the mosquito larvae were added to the plots. The report only states that the larvae were introduced ten times during the study. The registrant is required to provide this information.

Reviewer's Note: There is consistency between the two plots (both never fall below 90% EI) for up to 64 days, which concur with label efficacy claims. However, there is inconsistency in experimental data between the two RF2016 MT plots after day 64. Should the registrant wish to extend the time period of efficacy of the product in the future, a new efficacy study will have to be provided because of this disparity and insufficient replication of data.